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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 611 569 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number : **94300331.9**

(22) Date of filing : **18.01.94**

(51) Int. Cl.[5] : **A61K 31/23,** C07C 69/587, A23L 1/30

(30) Priority : **27.01.93 GB 9301582**
29.01.93 GB 9301801

(43) Date of publication of application :
24.08.94 Bulletin 94/34

(84) Designated Contracting States :
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

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AMENDED
SPECIFICATION
ORIGINAL
FILED BEHIND

(54) Triglycerides.

(57) A triglyceride for use in therapy or as a nutritional supplement, or a composition containing a triglyceride, wherein the triglyceride comprises a fatty acid selected from gamma-linolenic acid and the n-6 EPAs naturally derived therefrom and stearidonic acid and the n-3 EFAs naturally derived therefrom, forming a triple ester with glycerol or alternatively forming a double ester in which the other esterifying acide is a single residue of linoleic acid, with the proviso that the di-gammalinolenoyl-mono-linoleoyl glyceride if selected is used as a preparation containing more than 20% by weight thereof.

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Korrigierte Fassung / Corrected Version / Version Corrigée

Field of Invention

The invention relates to triglycerides.

Background

The essential fatty acids (EFAs) consist of a series of twelve compounds illustrated in Table 1 below. Although linoleic acid the parent compound of the n-6 series of EFAs, and alpha-linolenic acid the parent compound of the n-3 series, are usually the main dietary EFAs, these substances as such have relatively minor roles in the body. In order to be fully useful to the body, the parent compounds must be metabolized by the sequence of reactions shown in Table 1. In quantitative terms, as judged by their levels in cell membranes and in other lipid fractions, dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (AA) are the main EFA metabolites of the n-6 series, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main metabolites of the n-3 series. DGLA, AA, EPA and DHA are important constituents of most of the lipids in the body. As well as being important in themselves they can also give rise to a wide range of oxygenated derivatives, the eicosanoids, including the prostaglandins, leukotrienes and other compounds.

The elongation reactions shown in Table 1, in which 2 carbon atoms are added to the chain, tend to be rapid, whereas the desaturation reactions in which an extra double bond is introduced tend to be very slow. Thus for example gamma-linolenic acid (GLA) is rapidly converted to DGLA while stearidonic acid is readily converted to 20:4n-3 and so these pairs of compounds are equivalent in dietary terms. However, DGLA is only slowly converted to AA. None of the reactions are normally reversible, in man, nor are n-3 and n-6 series acids inter-convertible.

The table is as follows:-

molecule were to replace one of the GLAs in tri-GLA. This might possibly allow the enzyme to obtain better access to the triglyceride. We therefore synthesised tri-GLA using 14-C labelled GLA, and a triglyceride with two GLA molecules and one LA molecule, the GLA being labelled with C-14. We exposed the two triglycerides to pancreatic lipase for five minutes, isolated the remaining triglyceride and estimated the amount of radioactivity released. At the end of five minutes, in the case of tri-GLA just over 95 % of the radioactivity remained in the tri-GLA form, whereas in the case of di-GLA-mono-LA, only 88% of the radioactivity remained in the triglyceride form. Thus the rate of digestion of the compound containing one LA was more than twice as great as the pure tri-GLA. Di-GLA-mono-LA, while not as easily digestible as tri-LA, was more easily digestible than tri-GLA. The present invention thus comprehends triglycerides containing one linoleic acid and two molecules of a fatty acid which has undergone 6-desaturation as a way of delivering those fatty acids in a relatively digestible form.

Sources, Synthesis

Di-GLA-mono-LA (LGG) is a triglyceride found in substantial amounts in borage oil. The amounts vary depending on the source of the oil but are usually less than 10%. No special attention has ever been paid to LGG and there is nothing specific in any literature about its biological properties. As far as we are aware it is not found in any known preparation at a concentration higher than 20%. Preparations of such concentrations are therefore an aspect of the invention. Also as far as we are aware the other triglycerides described in this application are not found in nature at all. The ones of particular interest are triglycerides in which one fatty acid consists of linoleic acid and the other two are either GLA as above on the completely new compounds with DGLA, arachidonic acid, EPA or DHA.

These triglycerides may be prepared in several different ways by those skilled in the art. One approach is to purify the individual fatty acids and then to mix them in a ratio of two parts of the specific fatty acid with one of LA in a reaction vessel. The triglycerides may then be synthesised by a chemical reaction using, for example, zinc as a catalyst, or by the use of appropriate enzymes. The resulting mix of triglycerides can be used as such or, if appropriate, can be further purified by using techniques such as low temperature crystallisation, selective solvent extraction or the various forms of chromatography including high pressure liquid chromatography, to produce a mixture of TGs in which LGG predominates but in which there are smaller amounts of LLG, LLL and GGG. This mixture can be used itself or further purified. Similar techniques can be used for any of the triglycerides.

Broadly, the triglycerides may preferably be prepared as follows:

a) The individual fatty acids are purified from natural animal, vegetable or microbial sources or are chemically synthesized by methods known in themselves to those skilled in the art or by methods to be developed in the future. For example, particular fatty acids may be separated by such techniques as low temperature crystallisation, urea complex formation, silver complex formation, differential solubility and various forms of chromatography including high pressure liquid chromatography.

b) The individual fatty acids are then esterified with glycerol by chemical or enzymatic methods known in themselves to those skilled in the art or to be developed in the future. For example, the fatty acids and glycerol may be allowed to react together in the presence of one of a number of appropriate enzymes, or of p-toluene sulphonic acid hydrate.

c) If required, through the presence of undesired acids in the starting individual fatty acid, the specific triglycerides are further purified by appropriate methods known to those skilled in the art, in particular high pressure liquid chromatography or other appropriate forms of chromatography; low temperature crystallisation; or the use of solvents which differentially select triglycerides of particular composition.

Examples of Preparation

Example 1

Preparation of Tri-(z,z,z octadec-6,9, 12-trienoyl) glycerol (Tri-all cis GLA, Tri-GLA)

An example of the manufacture of tri-GLA is as follows:

1. Borage oil as a GLA-rich natural oil is saponified/hydrolysed to obtain the free fatty acids, GLA 8-25%.
2. The GLA is concentrated by two stages of urea crystallisation or low-temperature crystallisation to 45-50% GLA initally then to a product in which 70 to 95% of the material is GLA, reducing or eliminating the saturated, monounsaturated and diunsaturated fatty acids.
3. The fatty acid concentrate is distilled using short path distillation under vacuum to remove all the non

fatty acid material present (170°C/vacuum 10^{-2} mB).

4. Reverse phase HPLC is used to purify the GLA and collect the pure GLA fractions. The fatty acid mixture from stage 3 is dissolved in a mobile phase at 20% by weight . Appropriate mobile phases are mixtures of CH_3OH and water or CH_3CN and water. The stationary phase can be a monophasic C-18 reverse phase silica packing material. Detection is by a refractive index detector or by a UV detector reading at 210 or 215nM. A suitable HPLC system is a CEDI 1000 system fitted with two 10cm diameter cartridges. The GLA peak emerging from the HPLC system is collected.

5. Residual solvent is removed under vacuum and mild heat and any residual water by redissolving the product in a small volume of hexane and passing through anhydrous sodium sulphate. The final product is obtained by evaporation of the hexane, GLA 99% +.

6. Finally pure GLA from (5) is stirred and heated under vacuum to 140°C with a small stream of nitrogen passing through the liquid. To each 100g of GLA is added a solution of p-toluene sulphonic acid in warm glycerol (1.8g in 10.2g) over a period of 10 min. The mixture is then kept under these conditions for 6 hours, the water formed in the reaction being condensed out in an ice-cooled vessel. After cooling, the reaction mixture is purified by MPLC using a 500mm x 65mm diameter column packed with Matrex silica, pore size 60A, particle size 35-70μm. The solvent used is initally hexane, then 5% diethyl ether in hexane. The solvent is finally removed by distillation under vacuum to give tri-GLA as a pale yellow oil, 99.5%.

Other pure single fatty acid triglycerides may be made in a corresponding way.

Example 2

Preparation of di-(z,z,z octadec-6,9,12-trienoyl)-mono-(z,z octadec-9,12-dienoyl) glycerol (LGG)

By the method of the above example pure GLA is prepared, and correspondingly pure linoleic acid.

A 2:1 molar mixture of the free acids is then prepared and reacted with glycerol by the method of stage 6 of that example.

Finally preparative MPLC (medium pressure liquid chromatography) is applied to the mixture of LGG, LLG, LLL and GGG, again as in the example of preparation of GGG, to give the title compound as a clear oil, purity >99.5%.

The same method may be applied in the preparation of other glycerides with one residue of linoleic acid and two residues of a "6-desaturated" fatty acid other than gamma-linolenic acid. Equally for LGG itself the triglycerides of borage oil may be purified by fractionation followed by the application of MPLC to give the essentially pure material.

Example 3

Preparation of Tri-(z,z,z eicosa-8,11,14-trienoyl) glycerol (Tri-DGLA)

First, DGLA is prepared chemically from GLA as follows:-

Stage 1: z,z,z octadeca-6,9,12-trienyl methylsulphonate:- To a solution of z,z,z octadeca-6,9,12-trienol (175.5g) and dry pyridine (83ml) in dichloromethane (900ml) cooled to 0-5°C and under nitrogen was added methylsulphonyl chloride (121.6g) over a period of 30 minutes. The mixture was stirred for 48 hours at room temperature diluted with diethyl ether (4000ml) and the organic layer washed with 2M hydrochloric acid (400ml) and finally with brine (3 x 1000ml). After drying ($MgSO_4$), the solvents were removed *in vacuo* (50°C/20mmHg and 25°C/0.01mmHg) to give crude z,z,z octadeca-6,9,12-trienyl methylsulphonate (226g,99%) as a dark oil. This crude material was used for the next stage.

Stage 2: 2-(z,z,z octadeca-6,9,12-trienyl) propan-1,3 dioic acid:- To a solution of sodium ethoxide in absolute ethanol (from sodium, 15.3g, ethanol 500ml) was added diethyl malonate (118.7g). Over a period of 20 minutes and under nitrogen, was then added z,z,z octadeca-6,9,12-trienyl methylsulphonate (120g). The mixture was heated under reflux for 4 hours. After cooling, the resulting orange gelatinous mass was diluted with a solution of potassium hydroxide (150g) in water (76ml) and ethanol (1500ml), stirred under nitrogen at room temperature for 4 hours and then allowed to stand for 20 hours. The resulting precipitate was filtered off and dissolved in water (2000ml). The filtrate was evaporated *in vacuo* and the resultant oil added to the aqueous solution. Acidification (20% aqueous sulphuric acid) with cooling gave an oil which was extracted into diethyl ether (2 x 1000ml), the ether layer being washed with water (6 x 1000ml). Salt may need to be added to break up emulsions. Drying ($MgSO_4$) and evaporation of the solvent (30°C/20mmHg and 30°C/0.01mmHg) gave 2-(z,z,z octadeca-6,9,12-trienyl) propan-1,3-dioic acid(104.5g, 85%) as an oil which quickly solidified to a yellow low melting solid. This material was used for the next stage.

Stage 3: z,z,z eicosa-8,11,14-trienoic acid:- 2-(z,z,z octadeca-6,9,12-trienyl) propan-1,3-dioic acid (104.5g) was heated under vacuum (140° C/0.01mmHg) for 5 hours or until the production of carbon dioxide ceased. After cooling, the resulting dark oil was subjected to MPLC (Column size: 15mm die x 450mm, Column packing: Matrex silica, pore size 60A, particle size: 35-70µm, Solvent: Hexane, Fraction size: 500ml). Collection of the requisite fractions and removal of the solvent (50°C/20mmHg then 50° C/0.01mmHg) gave z,z,z eicosa-8,11,14-trienoic acid (68.3g, 74.5 %) as a clear oil.

Secondly, the DGLA is reacted with glycerol with subsequent purification, by the method of stage 6 of Example 1, to give the title tri-DGLA as a clear oil.

Uses

The specified triglycerides have a wide variety of possible uses. They may be used as pharmaceuticals for the treatment or prevention of diseases in which abnormalities of EFAs have been identified. They may be added to foods or be added to or used as nutritional supplements for those who require the particular EFAs for the treatment of prevention of diseases. They may also be used in foods or pharmaceuticals for veterinary use. They may be used for skin care.

Specifically, the triglycerides may be used in the form of an oil for addition to foods or skin care preparations or as a component of a pharmaceutical formulation for oral, topical or parenteral use. The oil used in preparing such foods, skin care products or pharmaceuticals should desirably contain more than 20%, preferably more than 40%, very preferably more than 60% and ideally more than 80% of the particular triglyceride of interest.

The triglycerides may be formulated in any way appropriate, as is well known to those skilled in the art of preparing pharmaceuticals, skin care products or foods. They may be administered orally, enterally, topically, parenterally (subcutaneously, intramuscularly, intravenously or otherwise), rectally, vaginally or by any other appropriate route.

Use Examples

The following are examples of modes of use of the triglycerides;

1. Any one of the specified triglycerides made up in soft or hard gelatin capsules of any size between 100mg and 1g and administered to provide a daily dose of between 100mg and 10g.
2. Any one of the specified triglycerides microencapsulated in gelatin or agar or any other appropriate material, or incorporated into any appropriate material to form a powder which can be taken orally, added to foods, tabletted, encapsulated, packed in sachets or any other appropriate form.
3. Any one of the specified triglycerides made up in a whip, liquid, cream or other appropriate form for oral administration.
4. Any one of the specified triglycerides made into a cream, ointment or other topical preparation at a concentration ranging from 0.1 to 30%.
5. Any one of the specified triglycerides made up into an emulsion suitable for parenteral administration.
6. Any one of the specified triglycerides added to any appropriate food material such as a spread, drink, candy, cereal, infant food or bakery product.
7. Any of the above may be used in conjunction with oleic acid as such or as its glyceride.
8. As 1 to 7 above, but wherein the glyceride has two residues of the same fatty acid, and one linoleoyl residue.

Claims

1. For use in therapy or as a nutritional supplement, a triglyceride comprising a fatty acid selected from gamma-linolenic acid and the n-6 EPAs naturally derived therefrom and stearidonic acid and the n-3 EFAs naturally derived therefrom, forming a triple ester with glycerol or alternatively forming a double ester in which the other esterifying acide is a single residue of linoleic acid, with the proviso that the di-gamma-linolenoyl-mono-linoleoyl glyceride if selected is used as a preparation containing more than 20% by weight thereof.

2. A pharmaceutical or dietary composition wherein one or more of the triglycerides specified in claim 1 forms more than 10% preferably more than 30% very preferably more than 70% and ideally more than 90% by weight of the triglycerides present.

3. A composition according to claim 2 made up to provide a daily dose of 1mg to 100g preferably 10mg to 10g and very preferably 500mg to 4g of the said triglyceride(s).

4. A composition according to claim 2 especially a dietary composition or skin care product, comprising the said triglyceride(s) in a concentration of 0.001 to 50% preferably 0.5 to 20% and very preferably 0.1 to 5% weight.

5. As such, the double esters specified in Claim 1, other than the di-gammalinolenoyl mono-linoleoyl glyceride.

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European Patent Office
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(11) EP 0 611 569 A3

(12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
30.07.1997 Bulletin 1997/31

(43) Date of publication A2:
24.08.1994 Bulletin 1994/34

(51) Int Cl.[6]: **A61K 31/23**, C07C 69/587, A23L 1/30

(21) Application number: **94300331.9**

(22) Date of filing: **18.01.1994**

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(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **27.01.1993 GB 9301582**
29.01.1993 GB 9301801

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(54) Triglycerides

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EUROPEAN SEARCH REPORT

Application Number
EP 94 30 0331

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
P,X	CLIN. SCI., vol.84, no.5, May 1993 pages 511 - 516 N. NAKAMURA 'Intravenous infusion of tridihomo-gamma-linoleoyl-glycerol reduces leukotriene B4 production in the rat and rabbit' * the whole document *	1-4	
X	EP-A-0 271 909 (GREEN CROSS CORPORATION) * the whole document *	1-5	
X	J. HIGH RESOLUT. CHROMATOGR., vol.15, no.4, 1992 K. AITZETMÜLLER 'Separation of highly unsaturated triacylglycerols by reversed phase HPLC with short wavelength UV detection.' * the whole document *	1-5	
X	EP-A-0 520 624 (EFAMOL HOLDINGS PLC) * page 5, line 35-38 *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.5)
E	WO-A-94 10125 (SANDOZ LTD.) * claims; examples 4,7 *	1-5	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 September 1994	Orviz Diaz, P

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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